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Inhibitory effect of chitosan oligosaccharides on the growth of tumor cells

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Introduction

Chitin, a poly β-(1→4)-N-acetyl-D-glucosamine, is best known as a cell wall component of fungi and as a skeletal materials of invertebrates. Chitosan is derived from chitin by deacetylation in the presence of alkali. Chitosan has been developed as new physiological materials since it possesses antibacterial activity, hypocholesterolemic activity and antihypertensive action. However, the actions of chitosan *in vivo* still remain ambiguous as the physiological functional properties because most animal intestines, especially the human gastrointestinal tract, do not possess enzyme such as chitosanase which directly degrade the β-glucosidic linkage in chitosan, and consequently the unbroken polymers may be poorly absorbed into the human intestine. Therefore, recent studies as chitosan have attracted interest for chitosan oligosaccharides, because the oligosaccharides process not only water-soluble property but also versatile functional properties such as antitumor activity, immuno-enhancing effects, enhancement of protective effects against infection with some pathogens in mice and antimicrobial activity (Kingsnorth et al., 1983, Mori et al., 1997).

In the present study, we investigated the effect of chitosan oligosaccharides on the rate of cell proliferation of EAT, A549, Hepa1c1c7, HeLa, Ac2F and Chang cells (Sladowski et al., 1993)

Materials and Methods

Chitosan oligosaccharides (COS) were donated by Kitto Life Co. EAT (mouse ascites carcinoma) cells were grown and maintained in NCTC-135 medium with 10% fetal bovine serum (FBS). Hepa1c1c7 (mouse hepatoma), HeLa (human epithelioid carcinoma, cervix) and Ac2F (rat normal liver cell) cells were grown and maintained in Eagle's minimum essential medium (MEM) with 10% FBS. A549 (human lung carcinoma) and Chang (human normal liver cells) cells were grown and maintained in Dulbecco's Modified Eagle's medium (DMEM) with 10% FBS. Cultures were maintained in 75-㎠ culture flasks at 37℃ in a 5% CO_2 atmosphere. After 2hr, 20 ㎕ of COS I (MW :

3,000-5,000) and COS II(MW : 1,000-3,000) was added with 0.1, 0.5 and 1.0 ㎎/㎖ and the culture cells were reincubated. After 72hr, MTT assay of cell viability carried out. Briefly, MTT was dissolved in PBS at 5㎎/㎖ and filtered to remove any insoluble residues. Twenty microtiters of MTT were addded to each well and incubated at 37℃ for a further 4hr. Plates were centrifuged at 450×g for 5min in a plate holder and then the media was aspirated from plates. Care was not to disturb the formazan crystals at the bottom of the wells. One hundred fifth microliters of 1:1 dimethyl sulfoxide (DMSO) and ethanol mixture were added to each well and the plates were placed on a shaker for 20min to solubilize the formazan crystals. The plates were then read immediately at 540nm on a scanning multiwell spectrophotometer (enzyme-linked immunosorbent assay reader ; Behring Co., Marburg, Germany). MTT assays were performed in triplicate.

Results and Summaries

Chitosan Oligosaccharides were shown to possess considerable toxicities toward various tumor cell lines. COS I (MW : 3,000-5,000) at the concentration of 1.0 mg resulted in approximately 66% inhibition of the growth of HeLa cells after 72 hours incubation. Toxicity of COS I to EAT, A549 and Hepa1c1c7 revealed a reduction in cell viability of 40.1%, 35.2% and 23.3%, respectively. Growth inhibition of COS II for A549, HeLa, Hepa1c1c7 and EAT was approximately 34.1%, 24.1%, 23.2% and 9.8%, respectively. However, there was no inhibition of the growth of normal cells, A_C2F and Chang, with the treatment of COS I or COS II. In morphological study, the number of cells was decreased, and the shape of cells was round in EAT, Hepa1c1c7, HeLa and A549 with the treatment of COS. But the morphology of A_C2F and Chang cells was normal. These results suggest that COS I and COS II have inhibitory effect on the growth of tumor cells but no inhibitory effects on the growth of normal cells.

References

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